THE ADHESIVE ORGANS OF AMIA.

ALBERT C. EYCLESHYMER AND JAMES MEREDITH WILSON.
From the Anatomical Laboratory of St. Louis University.

Most of the ganoid fishes have adhesive organs or sucking disks in the early stages of their development.

These organs have received little attention even from morphologists and systematists. Their development has not been worked out with any completeness in any species, but enough has been learned to raise numerous questions. The earliest stages in their development have never been described; even the germ layer from which they take their origin is unsettled. Their relations to other systems, digestive system, nervous system, etc., are in dispute. The various stages in their development, their structure during these stages and the functions they perform, as well as questions of their ultimate fate and their phylogeny and meaning seem to us to merit further study.

In a previous paper ('06) on the "Development of Amia" we have touched briefly on these organs. The present paper is a continuation of the same subject and is based on the same material.

Description of Stages.

The anlagen of the adhesive organs appear in the embryo about seventy hours after fertilization, or more precisely, when the embryo extends over about 160 degrees of the circumference of the egg. A median sagittal section of the anterior portion of an embryo of this stage is shown in Fig. 1. The ectoderm consists of a single layer of cells externally (*s.ec.*) and a large mass of deeper lying cells; the deeper layer is some 15 to 20 cells in thickness. In this mass of cells which forms the anlage of the future brain there is a slight cavity (*br.c.*) which is the first cavity to appear in the brain. In front of the brain is a second mass of thickened ectoderm (*p.cb.*) which has been called the precerebral mass. The gut-cavity (*g.c.*) is now widely extended laterally. In its anterior portion its dorsal and ventral walls are

closely apposed, yet they can be traced as distinct layers to a point immediately below the pre-cerebral mass. It is in this locality that the adhesive organs appear.

In an embryo of ninety hours which covers about 180 degrees of the circumference of the egg the adhesive organs are not observable in surface views but in sections their beginnings are obvious. Fig. 2 represents an obliquely sagittal section of the anterior portion of an embryo in this stage. The superficial ectoderm (*s.ec.*) consists of two layers of cells which are invaginated just at the anterior boundary of the forebrain (*f.b.*). The brain cavities are now well defined. There is, however, as yet no indication of the infundibular or epiphysial evaginations. The notochord extends nearly to the level of the anterior end of the brain, but owing to the obliquity of the section its anterior portion is not represented in the figure. The cavity of the gut is well defined beneath the posterior portion of the brain; it is greatly reduced in size anteriorly but after reaching the level of the ectodermic invagination described above, it again expands into a wide cavity (*g.d.*). The walls of the gut show little change until the head region is passed when the cells of the dorsal wall change from flat or cuboidal to columnar. These greatly thickened areas of the entoderm form the beginnings of the adhesive organs.

Transverse sections of this stage (Fig. 3) show that the organs have arisen as paired diverticula of the anterior end of the foregut.

In an embryo of one hundred and twenty-five hours, covering about 260 degrees of the circumference of the egg the adhesive organs show plainly in surface views as paired structures lying on either side of the median line. An obliquely sagittal section passing through one of the organs is represented in Fig. 4. It will be noted that the lumen of the brain is enlarged and its subdivisions more clearly marked. The dorsal wall of the forebrain now shows a slight evagination which is the beginning of the epiphysis; just opposite in the floor is another evagination which is the beginning of the infundibulum. The foregut is here well shown with its forward extension into the pre-cerebral region where it ends in a wide dilatation (*g.d.*). The dorsal wall of this cavity is composed of elongated entodermal cells essentially similar to those seen in Fig. 2. Just beneath the adhesive organ is one of

the lateral evaginations of the cœlom which later unites with a corresponding structure on the opposite side to form the heart.

Fig. 5 shows an obliquely transverse section through an embryo somewhat older. The anterior end of the forebrain (*f.b.*) appears as a solid mass of elongated cells. In connection with its ventral wall, the optic stalk passes obliquely outward and terminates in the optic vesicle (*o.v.*). On the other side the section passes through the anterior portion of the adhesive organ (*a.o.*) which here shows its connection with the anterior end of the foregut (*g.*). The foregut is almost closed off ventrally through the approximation of the cœlomic cavities. Between the end of the brain and the optic vesicles there is a slight invagination (*n.*) of the deep ectoderm to form the beginning of the nasal pit. The character of the epithelium of the adhesive organ is now more plainly shown owing to the absorption of yolk granules. It will be noted that the adhesive organ is now in contact with the superficial ectoderm (*s.ec.*) which extends over it as a double layer.

Another section through an embryo a few hours older taken in a sagittal plane is shown in Fig. 6. The section passes through the embryo in such a direction that one of the adhesive organs is cut lengthwise. The organ at this time is still in open connection with the gut. Its antero-dorsal surface follows closely the external contour of the embryo. The peripheral end of the organ thus extends both posteriorly and mesially following the course of least resistance. In many of the sections of this stage, or slightly later, a constriction is formed around the middle of each of the large organs which soon gives rise to a pair on each side of the snout.

The minute structure of the organ is very similar to that shown in Fig. 5. It is composed of very high columnar epithelium that presents a pseudo-stratified appearance. These columnar cells are heavily laden with yolk granules which are very large and dense at the bases of the cells but gradually diminish in density and size toward the inner ends of the cells until these ends become free from yolk. These yolk-free ends show that cellular metabolism is here most active. The indications are, from the condition of the reticulum and the stainable fine granules, that they are secreting cells. Moreover the presence of small droplets on the surface of the cells adds confirmation to this view.

The section of the organ represented in Fig. 7 is from a larva in which the head and tail are both free from the yolk.

The organs are now entirely detached from the gut although the two layers of the entoderm forming the gut diverticula are still discernible. They lie, for the most part, in the ectoderm. They have forced their way through the deeper layers and are covered externally by only a single layer. Even this layer is now very thin and the cell boundaries are no longer well defined. The two organs are now separated from each other and each is subdivided into six to ten separate disks. Fig. 7 represents a section through a single disk. The minute structure of the organ is similar to that previously described. It is composed of pseudo-columnar cells in which the nuclei are situated nearer the bases of the cells than hitherto noted. The basal half of each cell contains some large yolk granules and a coarse reticulum which takes a deep hæmatoxylin stain. The peripheral half of the cells, on the other hand, is free from yolk but contains a fine reticulum which stains only faintly with hæmatoxylin. This faintly staining portion of the cell contains minute granules which are best interpreted as prozymogen granules. Although the organ has all the appearances in the present stage, as it had in several of the preceding stages, of a mucous secreting structure it is not yet functional as an adhesive organ. One remarkable change has taken place in the structure of the organ. The entodermal cells show a changed polarity. In the preceding stages the most active cell metabolism was in the ends of the cells next to the gut cavity ; now the most active cell metabolism is in the outer ends of the cells next to the exterior of the body, while the earlier clearer ends are now filled with fine granules.

In the larva of 4–5 mm. the organs have broken through the superficial ectoderm and open directly on the surface of the body, although they are as yet partly covered by the ectoderm, as a glance at Fig. 8 will show. The cells of the organ here shown are not different from those described in the preceding stage excepting that the clear zone of the cell appears to extend over one third of its length instead of one half.

In the larva of 8–9 mm. (Fig. 9) the disk has changed from an oval to a rectangular form as a comparison of Figs. 8 and 9

will show. The ectoderm (*s. ec.*) has undergone considerable thickening and differentiation. It no longer extends over any portion of the organ and as a result a far greater number of cells now reach the surface. The character of the cells is somewhat different as a result of the general change in the form of the organ. They are now typical, pseudo-stratified columnar cells while in preceding stages the outer ends were compressed. The cells no longer possess yolk granules while the prozymogen granules show more plainly in the outer ends. That some sort of a mucous secretion is furnished by these cells is indicated, not only by the cellular structure but also by the fact that when the larvæ are detached from the various objects, to which they now adhere, it is often observed that fragments of aquatic plants cling to the organs.

The larva of 13–14 mm. begins to move freely from place to place. It rarely attempts to attach itself as in the preceding stages. Corresponding with this change in the behavior of the young fish there is a marked change in the position of the adhesive organs. When viewed from the surface, some of the disks forming the horseshoe are still distinctly visible while others are barely discernible. All, however, show a great reduction in number when compared with the larva of 8–9 mm. A section of one of these organs is shown in Fig. 10. It will be noted that the entire organ has now sunk below the level of the epidermis and is partly surrounded by dermal pigment. The organ is still in communication with the exterior through a funnel-shaped opening in the epidermis.

In the larva of 18–20 mm. the organs have sunk still deeper below the level of the epidermis. Some of them still communicate with the exterior, while others are completely covered by the epidermis. Those that communicate with the exterior show a long narrow epithelial tube with numerous branches or diverticula at its inner end. The walls of these tubes are several layers of cells in thickness as shown in Fig. 11. Toward the inner end of the tube the epithelium becomes thinner until there remains but a single layer of cells covering the smaller branches. This layer becomes broken up in the smallest branches and these epithelial cells become swollen, the nuclei show chromato-

lysis and the cells are absorbed. The ectodermal cells forming the organs, as shown in the figure, likewise are much swollen, their outlines are indistinct and connective tissue grows in among them. Their nuclei are less readily stained with basic stains. The cytoplasm becomes vacuolated and granular; all of which points to albuminoid degeneration.

Historical and Critical.

The first author to treat of the development of the adhesive organ of any ganoid was Alexander Agassiz ('78) in his paper on *Lepidosteus*. But his observations were limited to the period after hatching. At this time the development of the organ is nearly completed and his descriptions therefore refer mainly to its function and its degeneration and disappearance. He describes the "huge mouth cavity" of the newly hatched *Lepidosteus* "surmounted by a hoof-shaped depression edged with a row of protuberances acting as suckers" and compares it with the mouth of the cyclostomes. The moment the little fish is hatched it attaches itself firmly to the side of the dish and there remains hanging immovable. Three days after hatching the disk becomes more prominent, "the individual suckers projecting frequently beyond the general outline of the edge of the suckers." As the snout lengthens the suckers become concentrated and the size of the terminal disk is reduced. When the fish is three weeks old "the sucking snout is now reduced to a swelling of the extremity of the elongated upper jaw." Later the disk is reduced to a single row of small suckers and finally it becomes the fleshy globular termination of the upper jaw of the adult.

Balfour and Parker ('82) continued the work on *Lepidosteus*, working on material furnished by Agassiz. They state that the disk is formed two or three days before hatching but give no details. They give two figures showing something of its histological structure and add: "The result of our examination has been to show that the disk is provided with a series of papillae often exhibiting a bilateral arrangement. The papillae are mainly constituted of highly modified cells of the mucous [inner] layer of the epidermis [epiblast?]. These cells have the form of elongated columns, the nucleus being placed at the base and the main

mass of the cells being filled with a protoplasmic reticulum. They may probably be regarded as modified mucous cells." In regard to its function they say: "It does not appear probable that the disc has a true sucking action. It is unprovided with muscular elements, and there appears to be no mechanism by which it could act as a sucking organ. We must suppose, therefore, that its adhesive power depends upon the capacity of the cells composing its papillae to pour out a sticky secretion."

Von Kupfer ('93) has described the development of the head in *Acipenser* basing his studies on median sagittal sections. He derives the adhesive organ from the inner layer of the ectoderm and states that it arises in close connection with the hypophysis. It is at first single but becomes paired by a groove on the median line. Each of these disks divides again, forming four papillæ. These four papillæ develop into the four barbels of the adult fish.

In regard to his derivation of the adhesive organs from the ectoderm we may say: (1) Kupfer's work is based on median sagittal sections and such sections are not those best adapted to trace out the development of a paired organ like the adhesive organs. (2) He has not described the earliest stages. His youngest stage is an embryo forty-five hours after fertilization. (3) Even by Kupfer's showing these organs are connected with the digestive system, though it may be only through the hypophysis.

Ehrenbaum ('94), as stated by Ziegler ('02, p. 157), gives a different origin for the adhesive organs in *Acipenser*. We translate from Ziegler: "On the under side of the head in front of the mouth one finds on each side two thickenings (Wülste) which become the barbels. At this place we noticed in a somewhat earlier stage a depression marked by a peculiar pigmentation; this corresponds to the sucking disk of *Lepidosteus* and *Amia*." How this is to be reconciled with von Kupfer's account of a common anlage for adhesive organs and hypophysis is not apparent to us. Unfortunately Ehrenbaum's paper is not accessible and we are not in position to give any further account of his work.

On the whole then, while we have the high authority of von Kupfer in favor of the view that in *Acipenser* the adhesive organs take their origin from the ectoderm, in our opinion the subject deserves further investigation as the proof is far from being conclusive.

Dean ('96) was the first author to treat of the development of the adhesive organs in *Amia*. His description begins with an embryo 138 hours after fertilization, that is, about two days before hatching. At this time the organ is already well defined; he notes its paired character and states that at hatching it is relatively at its largest size. Most of his observations have reference to its degeneration and disappearance. This process begins in a few days after hatching. By the seventh day the organ "is reduced to a mere tubercle and is no longer functional." On the tenth day "it is greatly reduced, although it does not in fact disappear entirely (histologically) for several weeks. . . . Its atrophy takes place first proximally, later marginally; the cells of its deepest tissues become greatly vacuolated and form a sponge-like mass and the cell wall which here forms its anterior boundary gradually encroaches; the cells of the centro-distal region are the last to retain their early character."

In his general discussion of his observations he homologizes the adhesive organs with "the typical pit organs, or sense buds which later occur on other integumental regions" and uses this homology as "evidence of how precociously embryonic and larval structures may be developed." Needless to say we find no basis for any such homology and no grounds for mentioning the subject of precocity.

In 1899 Miss Jessie Phelps working in Reighard's laboratory published a brief article on the "Development of the Adhesive Organs of *Amia*." This writer says: "The organ is formed in a very early stage as a diverticulum of the foregut. This diverticulum subsequently divides into two, each of which continues to communicate for a time with the cavity of the foregut. Each of the two diverticula later separates from the foregut, becomes elongated and curved into the form of a semicircle and divides into from six to eight closed vesicles. The vesicles finally open to the exterior and are thus converted into cups. After being functionally active for a time the organ is pushed beneath the surface by the thickening ectoderm, becomes infiltrated with leucocytes and finally disappears (larvæ of 20–25 mm.) without leaving any trace behind it."

The results of our work confirm the findings of Miss Phelps as

to the germ layer from which these organs are formed. But we cannot agree with her statement that the organ consists at first of a single diverticulum which subsequently divides into two. We find the anlagen of the adhesive organs in paired thickenings of the dorso-lateral portion of the anterior extremity of the fore-gut (Fig. 3). As soon as these become recognizable as diverticula there are plainly two of them.

As to their method of disappearance we are unable to find anything which indicates an infiltration of leucocytes or phagocytosis. On the other hand everything suggests an autogenous degeneration.

It has been shown beyond question that in *Amia* the adhesive organs are developed from the entoderm. But in the other ganoids, *Acipenser* and *Lepidosteus* — while the proof is not conclusive — the balance of authority favors the view that they are formed from the ectoderm. *A priori* it does not seem probable that homologous organs would be formed from different germ layers. But what proof have we that these organs are homologous? Sucking disks are formed in many species of animals in widely different groups. Generally they are temporary larval organs, and it can be easily shown that they are not in all cases homologous organs. In those amphibia which have sucking disks these organs are undoubtedly ectodermal in origin. In the teleost *Echeneis* (*Remora*) the great sucking disk — an adult organ in this case — is developed from the anterior end of the dorsal fin. Ascidians also develop sucking disks; they too are ectodermal.

As to the function of these organs all authorities seem to be agreed. Their function is to attach the young fish to some solid object. As a rule the organ is well developed when the fish is hatched and the little fish attaches itself immediately. This is true at least of *Lepidosteus* and *Amia*. In *Amia* the organ serves to keep the young fish attached for about a week; in *Lepidosteus* about two weeks.

Whether the organ has now or ever has had any other function has not been shown. It has been suggested that they may serve to convey some sort of nutriment to the digestive tract but we have found no proof of this. It has also been suggested that

they are modified sense buds but this is certainly not the case in *Amia*. But the fact that in *Acipenser* the adhesive organs become tactile organs, put with von Kupfer's statement that the adhesive organs and the hypophysis have a common anlage may suggest some remote connection with the nervous system.

As to the phylogeny or meaning of these organs facts are too few to form a basis for any very extensive or certain conclusions. In the various species of ganoids they may have a common function; whether they have a common origin is doubtful. They do not have a common fate. In *Amia* they disappear entirely. In *Lepidosteus* they leave only a useless rudiment. In *Acipenser* they become the barbels. This last fact may furnish a clue to the origin of the barbels in the teleosts as suggested long ago by Balfour. The adhesive organs of ganoids — if we may base our conclusions on their development in *Amia* — are not homologous with the sucking disks of the amphibia or the teleosts. So far as we can see now these organs were developed independently in different groups of animals. But living forms of ganoids are so few and so aberrant that any conclusions based on the study of these forms alone will probably be only tentative.

Summary.

The development of the adhesive organs in *Amia* begins about 70 hours after fertilization when the embryo extends over about 160 degrees of the circumference of the egg.

They appear as paired thickenings of the dorso-lateral portion of the anterior end of the foregut. They are therefore entodermal in origin.

They grow out as paired diverticula of the foregut, break through the epidermis, become greatly subdivided and form a horseshoe-shaped structure at the end of the snout.

Histologically they consist of high columnar epithelial cells. These cells are at first heavily laden with yolk granules especially at their base. They contain a reticulum coarser at the base. Metabolic activity is at first greater at the basal end of the cells but later it is greater at the distal end.

The function of these cells is undoubtedly to secrete a mucous substance by means of which the young fishes attach themselves

to plants or other fixed bodies. They function as secreting organs till they sink below the epidermis.

The organs reach the highest stage of their development just after hatching. They are functional for about a week and disappear in two or three weeks. The mode of disappearance is probably by cytolysis.

LITERATURE.

Agassiz, Alexander.
'78 The Development of Lepidosteus. Proc. Am. Acad. Arts and Sc. Boston, Vol. 13, p. 65-76.

Balfour, F. M.
'85 Treatise on Comparative Embryology. London, Vol. 2, p. 108, footnote.

Balfour, F. M., and Parker, W. N.
'82 On the structure and development of Lepidosteus. Phil. Tr. London, Vol. 175, pp. 359-442.

Dean, Bashford.
'96 On the larval development of Amia calva. Zool. Jahrb. Abt. f. Syst., Vol. 9, pp. 639-672, Taf. 9-11.

Ehrenbaum, Ernst.
'94 Beiträge zur Naturgeschichte einiger Elbfische (Stör). Wiss. Meeresuntersuch., Bd. 1, Heft. 1, Kiel und Leipzig.

Eycleshymer, A. C., and Wilson, J. M.
'06 The gastrulation and embryo formation in Amia calva. Am. Jour. Anat., Vol. 5, pp. 133-162, 4 pl.

Kupfer, C. von.
'93 Studien zur vergleichenden Entwicklungsgeschichte des Kopfes. Heft 1. Die Entwicklung des Kopfes von Acipenser Sturio. München.

Phelps, Jessie.
'99 The development of the adhesive organs of Amia. Science, N. S., Vol. 9, p. 366.

Salensky, W.
'82 Recherches sur le developpement du Sterlet (Acipenser ruthenus). Archives de Biol., Tome 2.

Ziegler, H. E.
'02 Lehrbuch der vergleichenden Entwickelungsgeschichte der niederen Wirbeltiere, Jena.

ABBREVIATIONS.

a.o.	adhesive organs.	*g.en.*	gut entoderm,
br.c.	brain cavity.	*h.b.*	hind brain.
c.	cœlom.	*ht.*	heart.
ch.	notochord.	*mes.*	mesoderm.
d.ec.	deep ectoderm.	*n.*	nasal pit.
en.	entoderm.	*o.v.*	optic vesicle.
f.b.	fore brain.	*p.*	pigment.
g.	gut.	*p.cb.*	pre-cerebral mass.
g.c.	gut cavity.	*s.ec.*	superficial ectoderm.
g.d.	gut diverticulum.	*y.m.*	yolk mass.

Explanation of Plate VI.

Figures 1–6 are magnified about 50 diameters; figures 7–11 about 450 diameters.

Fig. 1. Median sagittal section of the anterior portion of an embryo about seventy-two hours after fertilization, showing the anlage of the adhesive organs.

Fig. 2. Oblique sagittal section of anterior portion of embryo about ninety-five hours after fertilization.

Fig. 3. Transverse section through an embryo of same age, showing paired diverticula of the foregut which become the adhesive organs.

Fig. 4. Sagittal section of an embryo one hundred and ten hours after fertilization showing median portion of the adhesive organs and the heart.

Fig. 5. Obliquely transverse section of an embryo one hundred and twenty-five hours after fertilization passing through the anterior margin of the adhesive organ on one side and the optic stalk and vesicle on the other.

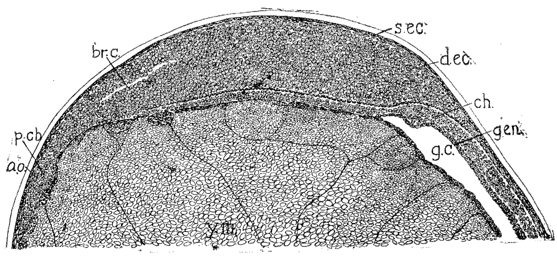

Fig. 1

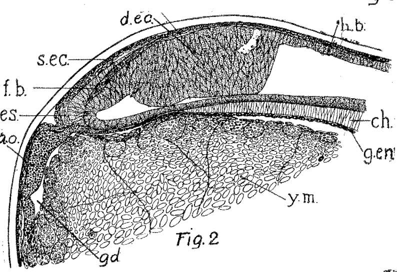

Fig. 2

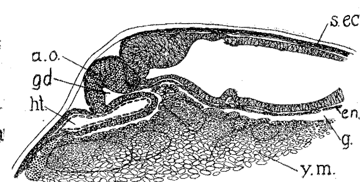

Fig. 4

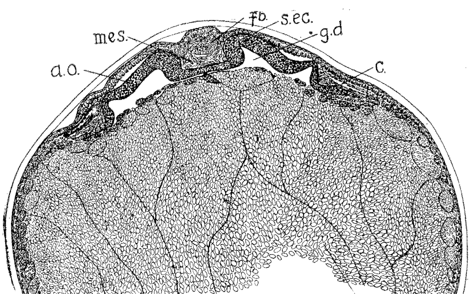

Fig. 3

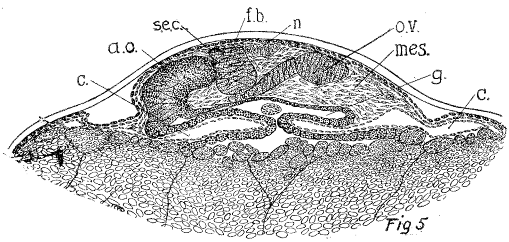

Fig. 5

A. B. Streedain, del.

EXPLANATION OF PLATE VII.

FIG. 6. Sagittal section through an embryo a few hours older than that shown in Fig. 5, showing one of the adhesive organs cut lengthwise.

FIG. 7. Section through a single disk of the adhesive organ of a larva in which both head and tail are free from yolk.

FIG. 8. Section through a single disk of a larva about 8 mm. in length.

FIG. 9. Section through a single disk of a larva about 9 mm. in length.

FIG. 10. Section through a single disk of a larva about 14 mm. in length.

FIG. 11. Section through a single disk af a larva about 20 mm. in length.

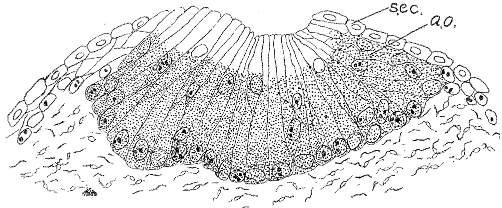

Fig. 8

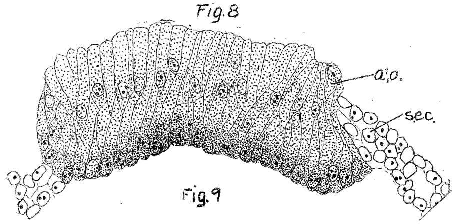

Fig. 9

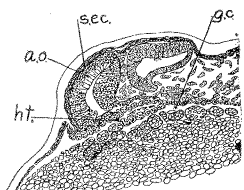

Fig. 6

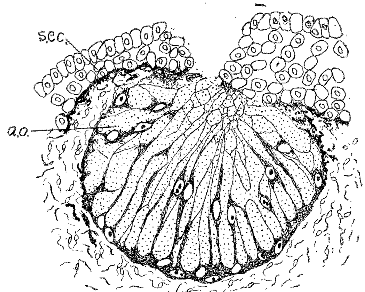

Fig. 10

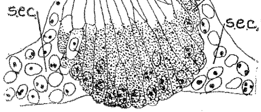

Fig. 7

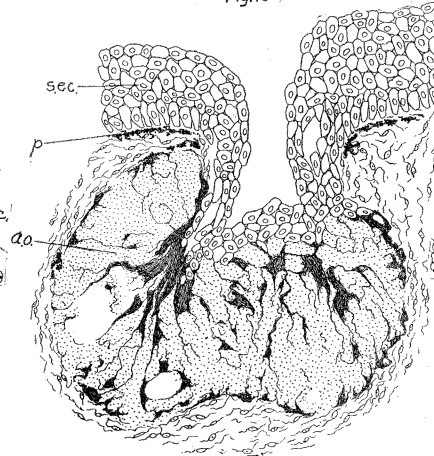

Fig. 11